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Metabolism of Alachlor and Propachlor in Suspensions of Pretreated Soils and in Samples from Ground Water Aquifers

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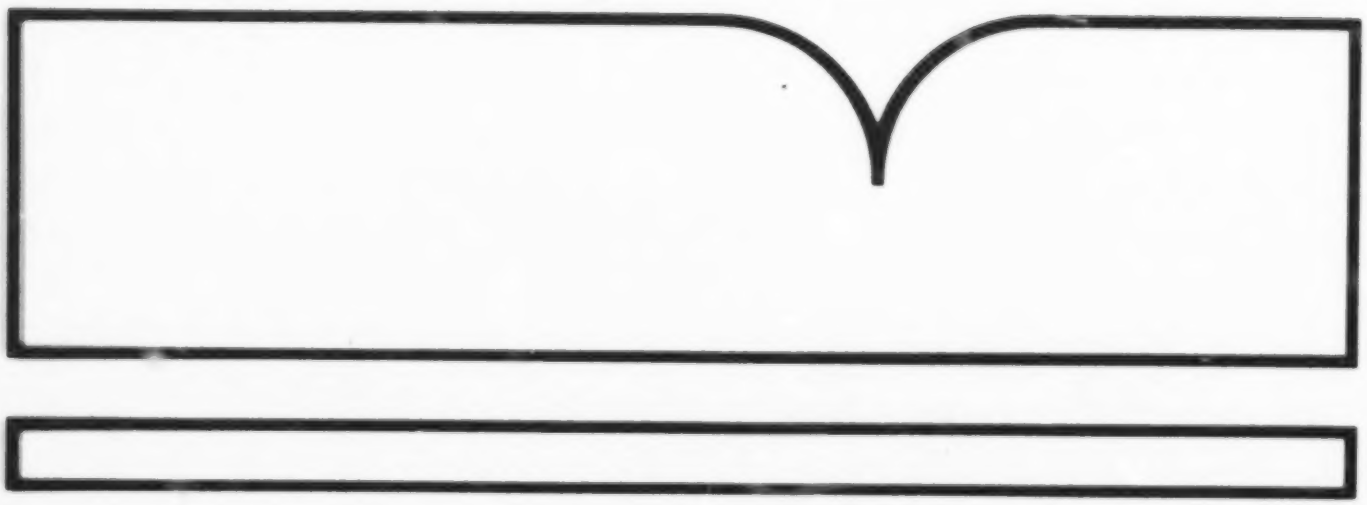

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16. ABSTRACT

Suspens[illegible] soils treated in the field with alachlor [2-chloro-2'[illegible]yl-N-(methoxymethyl)acetanilide] and propachlor (2-chlor-N-isc[illegible] acetanilide) were tested for their ability to metabolize these herbicides. Less than 8% of ^{14}C ring-labeled alachlor was mineralized in 30 days at concentrations of 10 and 0.073 µg/mL. The soil suspensions mineralized 16-61% and 0.6-63% of ring-labeled propachlor in 30 days at a concentration of 0.025 and 10 µg/mL of suspension, respectively. Although soils converted alachlor to organic products, microorganisms able to mineralize the pesticide could not be isolated. Samples from ground water aquifers mineralized less than 1% of the herbicides at the lower concentrations, but four organic products were formed from alachlor. A mixture of two bacteria mineralized 57.6% of ring-labeled propachlor in 52.5 h. A product of the microbial metabolism of propachlor was identified as N-isopropylaniline. These findings suggest that mineralization is a major means for the destruction of propachlor but not for alachlor in soil.

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Metabolism of Alachlor and Propachlor in Suspensions of Pretreated Soils and in Samples from Ground Water Aquifers

Norman J. Novick, Reba Mukherjee,[1] and Martin Alexander*

Suspensions of soils treated in the field with alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide] and propachlor (2-chloro-*N*-isopropylacetanilide) were tested for their ability to metabolize these herbicides. Less than 8% of ^{14}C ring-labeled alachlor was mineralized in 30 days at concentrations of 10 and 0.073 μg/mL. The soil suspensions mineralized 16–61% and 0.6–63% of ring-labeled propachlor in 30 days at a concentration of 0.025 and 10 μg/mL of suspension, respectively. Although soils converted alachlor to organic products, microorganisms able to mineralize the pesticide could not be isolated. Samples from ground water aquifers mineralized less than 1% of the herbicides at the lower concentrations, but four organic products were formed from alachlor. A mixture of two bacteria mineralized 57.6% of ring-labeled propachlor in 52.5 h. A product of the microbial metabolism of propachlor was identified as *N*-isopropylaniline. These findings suggest that mineralization is a major means for the destruction of propachlor but not for alachlor in soil.

Alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)-acetanilide] and propachlor (2-chloro-*N*-isopropylacetanilide) are preemergence herbicides used primarily to control grassy weeds. Microbial degradation has been reported to be the major means of destruction of chloroacetanilide pesticides in soils (Beestman and Deming, 1974). ^{14}C ring-labeled and carbonyl-labeled propachlor and alachlor have been reported to be not mineralized in soil (Lee et al., 1982; Chou, 1977), sewage, and lake water (Novick and Alexander, 1985). The degradation of alachlor by the soil fungus *Chaetomium globosum* has been extensively studied, and a number of metabolites have been identified (Tiedje and Hagedorn, 1975). Chou (1977) found that 2-chloro-2′,6′-diethylaniline and 1-(chloroacetyl)-2,3-dihydro-7-ethylindole was produced in soil from alachlor. Pathways of propachlor metabolism in plants and animals involving conjugation with sulfur-containing compounds have been described (Bakke et al., 1980; Lamoureux et al., 1971). Lee et al. (1982) identified a number of metabolites produced from propachlor in soil suspensions.

The purpose of the present study was to determine whether suspensions of soils that had received field applications of propachlor or alachlor would mineralize these compounds and whether samples from ground water aquifers, into which these compounds may leach, would metabolize them. Attempts were also made to enrich for and isolate microorganisms capable of mineralizing the ring carbons of alachlor and propachlor.

MATERIALS AND METHODS

Chemicals. Uniformly ^{14}C ring-labeled and analytical grade propachlor and alachlor were provided by Monsanto Co., St. Louis, MO. The labeled compounds had radiochemical purities of 97% as determined by thin-layer chromatography and specific activities of 7.1 and 18.9 μCi/mg for alachlor and propachlor, respectively.

CO_2 Evolution. The soils obtained from Cornell University experimental farms had received commercial preparations of alachlor (Lasso) and propachlor (Ramrod) at a rate per application of 4.7 L/ha and 6.7 kg/ha, respectively. Lima Kendaia silt loam, Munson very fine sandy loam, and Pittsfield fine sandy loam received a single application of alachlor in June 1984. Darien gravelly silt loam received single applications of alachlor in June 1983 and June 1984. Edwards muck soils received three or more applications of propachlor during each of the 1982, 1983, and 1984 growing seasons. The soils were collected in September and October, 1984, and passed through a 4-mm sieve. Aquifer material, which was a fine sand containing ground water collected aseptically as described by Wilson et al. (1983), came from a depth of 5 m from core 9Q2 at the U.S. Environmental Protection Agency field site in Lula, OK.

One gram of soil or 2 g of aquifer material and 10 mL of distilled–deionized water (Milli-Q Systems, Millipore Corp., Bedford, MA) were added to 100-mL serum bottles. To these suspensions was added an aqueous solution containing 730 ng of ^{14}C-labeled alachlor or 250 ng of labeled propachlor (10000 dpm), or the labeled chemical plus 100 μg of unlabeled herbicide. The bottles were sealed with Teflon-coated silicon closures and incubated at 28 °C on a rotary shaker operating at 100 rpm. Controls were autoclaved for 30 min. Duplicate bottles were used for autoclaved samples, and triplicate bottles were used for samples that were not autoclaved. At the end of the test period, samples were acidified with H_2SO_4, the resultant CO_2 was collected in KOH traps, and the radioactivity was determined as described by Novick and Alexander (1985) using a Beckman LS7500 liquid scintillation counter (Beckman Instruments, Fullerton, CA). The data presented represent the difference in radioactivity between samples that were not and that were autoclaved. The radioactivity found in the KOH traps of the sterile samples ranged from 0.5 to 2.5% of that added to the soil slurries.

Alachlor Metabolism. Ten grams of Darien soil or aquifer material and 10 mL of distilled–deionized water were added to 125-mL cotton-stoppered Erlenmeyer flasks. Controls were autoclaved for 1 h. Each flask received 1.5 μg of labeled alachlor. An additional 100 μg of unlabeled alachlor was added to the soil samples. The samples were incubated at 28 °C on a rotary shaker operating at 100 rpm.

After 45 days of incubation, the contents of duplicate flasks containing soil that was or was not autoclaved were poured into 50-mL centrifuge tubes, and residual material in the flasks was removed with three 5-mL portions of distilled–deionized water. The samples were centrifuged at 10000*g* for 10 min, and the aqueous phase was decanted and extracted twice with 50 mL of ethyl acetate. The soil

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material was placed in 500-mL Erlenmeyer flasks and extracted overnight with 100 mL of hexane–acetone (9:1) on a rotary shaker operating at 100 rpm. The samples were then filtered through Whatman No. 1 filter paper (Chou, 1977). To determine percent recovery of label, 4 mL of the organic fractions and 2 mL of the aqueous fractions were added in duplicate to scintillation vials with 8 and 12 mL of Liquiscint (Diagnostic Laboratories, Somerville, NJ), respectively, and the radioactivity was determined. By this method, more than 90% of the alachlor added initially was recovered after 1–2 h of incubation in the soil. The ethyl acetate and hexane–acetone fractions were dried over Na_2SO_4, concentrated to about 5 mL in a rotary evaporator operating at 38 °C, and then taken to dryness at 38 °C under N_2. The samples were resuspended in a small volume of ethyl acetate or hexane–acetone (9:1) and spotted on silica gel sheets (Eastman Kodak, Rochester, NY). Samples were run in a solvent system of toluene–methanol (95:5). Spots were located by autoradiography with Kodak X-OMAT AR film.

After 47 days of incubation, the contents of duplicate flasks containing autoclaved or unautoclaved aquifer samples were transferred to 50-mL centrifuge tubes with three 5-mL washes of distilled–deionized water, and the material was centrifuged at 10000*g*. The solids were then reextracted with an additional 25 mL of distilled–deionized water, and 2.0 mL of each aqueous extract was transferred in duplicate to scintillation vials and counted.

Alachlor and the products of its metabolism found in the aqueous soil extract (after extraction with ethyl acetate) and pooled aqueous fractions from the aquifer material were separated and quantified on C_{18} Sep-pak columns (Waters Associates, Milford, MA) as previously described (Novick and Alexander, 1985). The C_{18} columns removed more than 99% of the radioactivity from the aqueous samples. No counts remained on the column after elution with combinations of hexane, ether, and methanol. In experiments with aquifer samples, C_{18} column eluates were also examined by thin-layer chromatography. Fractions containing radioactivity that were eluted with hexane–ether were pooled separately from fractions containing radioactivity that were eluted with ether–methanol. The pooled fractions were taken to dryness under N_2 at 38 °C and then dissolved in a small volume of ether or methanol. Each sample was spotted on two silica gel thin-layer sheets, and one was developed in a solvent system of toluene–methanol (95:5) and one in acetonitrile–water–ammonium hydroxide (44:9:1). The spots were located by autoradiography.

Isolation of Propachlor and Alachlor Mineralizers. Portions (0.5 g) of the six soils were added together to 500-mL flasks containing 100 mL of an inorganic salts solution (Novick and Alexander, 1985) amended with 0.1 or 10 mg of propachlor or alachlor. Some flasks also received 25 μg of $CuSO_4 \cdot 5H_2O$, 200 μg of $MnCl_2 \cdot 4H_2O$, 200 μg of $ZnSO_4 \cdot 7H_2O$, 10 μg of $Na_2MoO_4 \cdot 2H_2O$, 2.0 μg of $CoCl_2 \cdot 6H_2O$, 10 μg of H_3BO_3, 20 μg of biotin, and 100 μg each of thiamine, calcium pantothenate, nicotinamide, and pyridoxine hydrochloride. After 14 days of incubation at 28 ° C on a rotary shaker, 1 mL of each enrichment was transferred to 10 mL of fresh medium of the same composition. Thereafter, transfers into fresh medium were made every 1–2 weeks for 6 months. Periodically, 0.1-mL portions of the enrichments were transferred into 10 mL of fresh medium containing 10000 dpm of ^{14}C-labeled propachlor of alachlor. After 1 week, the liquids were acidified with H_2SO_4 and bubbled with air for 10 min, and 1.0-mL portions were transferred in duplicate to scintillation vials containing 12 mL of Liquiscint and counted. Disappearance of the parent herbicide was determined by extracting unlabeled samples twice with 10 mL of ether, removing the water with Na_2SO_4, and drying the pooled ether extracts under N_2 at room temperature. The samples were dissolved in 0.2 mL of methanol and injected into a Perkin-Elmer 3920B gas chromatograph. The 2 mm × 183 cm stainless-steel column was packed with 3% OV-1 on 100/120 Gas Chrom Q (Applied Science Laboratories, State College, PA). The temperature of the column was maintained at 120 °C for 2 min and then increased at 8 °C/min to 220 °C. The injector and detector were at 230 °C, and the flow rate of the carrier gas (N_2) was 30 mL/min. Enrichments showing activity were streaked on half-strength Trypticase soy agar (Difco).

Product Identification. Portions (0.2 mL) of the enrichment culture were added to 25 mL of inorganic salts solution amended with 100 μg of propachlor/mL. After 42 h of incubation on a rotary shaker, the contents of triplicate 125-mL flasks and uninoculated controls were pooled separately, acidified with H_2SO_4, and extracted with 150 mL of ethyl acetate. The samples were adjusted to pH greater than 10 with KOH, and they were then extracted again with 150 mL of ethyl acetate. The extracts were pooled, dried with Na_2SO_4, and taken to dryness at 38 °C in a rotary evaporator. The samples were resuspended in 0.3 mL of pyridine and analyzed by gas chromatography on 3% OV-101 on 100/120 Gas Chrom Q (2 mm × 152 cm glass column). The operating conditions were the same as above except that the maximum column temperature was 300 °C and the oven temperature was increased at 4 °C/min. The products separated by gas chromatography were subjected to electron impact analysis using a Finnigan 3300 quadrupole mass spectrometer with a Systems Industries 150 data system.

RESULTS

Mineralization in soil suspensions was tested on soils that had received alachlor or propachlor in the field for one or more growing seasons. In five of the soils, less than 4% of the ring-labeled carbons of alachlor was converted to CO_2 in 30 days at alachlor concentrations of 73 ng/mL and 10 μg/mL of suspension (Table I). On the other hand, 7.3% of the alachlor at the lower concentration was mineralized in Lima-Kendaia silt loam. In contrast, all soil suspensions tested converted more than 15% of the ring carbons of propachlor to CO_2 in 30 days at one or both of the concentrations. In the Darien soil, the percentage mineralized was markedly greater at the higher propachlor concentration. Only 0.6% of the propachlor at the higher concentration was mineralized in Pittsfield fine sandy loam, but 16.2% was mineralized at the lower level. Suspensions of the two samples of Edwards muck, which had been pretreated with propachlor for 3 years, showed markedly more mineralization of propachlor than three of the other four soils. Of the soils not receiving propachlor in the field, comparable mineralization was only evident in suspensions of the Darien soil and only at 10 μg/mL. Less than 1% of the herbicides was mineralized in 30 days in the aquifer samples.

After 47 days of incubation of the aquifer samples with 150 ng of labeled alachlor/mL, 90.9 and 85.4% of the radioactivity were recovered from aqueous extracts of the unautoclaved and autoclaved samples, respectively. The alachlor in the samples was separated from products formed from it by means of a C_{18} column. In the sterile sample, 95.2% of the radioactivity placed on the column was recovered in fractions 1 (100% hexane) to 5 (60% hexane–40% ether), whereas in the nonsterile sample

Table I. Mineralization of Propachlor and Alachlor in Soils and Aquifer Material

environmental sample	chemical	seasons treated[a]	herbicide concn,[b] μg/mL	% mineralized
Darien gravelly silt loam (pH 6.1)	alachlor	2	10	2.2
			0.073	3.2
	propachlor	0	10	62.2
			0.025	20.5
Edwards muck (pH 5.4)	alachlor	0	0.073	0.2
	propachlor	3	10	63.3
			0.025	57.1
Edwards muck (pH 5.8)	alachlor	0	0.073	0.2
	propachlor	3	10	54.5
			0.025	61.0
Lima-Kendaia silt loam (pH 7.6)	alachlor	1	10	2.7
			0.073	7.3
	propachlor	0	0.025	32.5
Munson very fine sandy loam (pH 7.3)	alachlor	1	10	1.8
			0.073	3.5
	propachlor	0	0.025	25.0
Pittsfield fine sandy loam (pH 6.4)	alachlor	1	10	1.8
			0.073	2.7
	propachlor	0	10	0.6
			0.025	16.2
aquifer material	alachlor	NA[c]	0.073	0.5
	propachlor	NA	0.025	0.8

[a] Number of seasons during which the soil received the herbicide. Untreated soil designated as 0. [b] Concentration used for test of mineralization. [c] Not applicable.

60.6% of the ^{14}C was recovered in fractions 1 to 5 and 26.8% was in fractions 12 (90% ether–10% methanol) to 17 (40% ether–60% methanol) (Figure 1A). The hexane–ether and ether–methanol fractions containing ^{14}C were separately pooled, and the pooled fractions were spotted on silica gel plates and developed in toluene–methanol (95:5) or acetonitrile–water–ammonium hydroxide (44:9:1). Only alachlor was present in the hexane–ether fractions prepared from the autoclaved and unautoclaved samples. Products of alachlor metabolism were present in ether–methanol fractions, but alachlor was not found in these fractions. Components in the pooled ether–methanol fractions did not migrate from the origin when developed in toluene–methanol, but four spots were evident in the acetonitrile–water–ammonium hydroxide solvent system. They had R_f values of 0.41, 0.45, 0.51, and 0.55, and these products of alachlor metabolism represented 19.7, 10.2, 8.3, and 61.8%, respectively, of the ^{14}C in the ether–methanol fractions.

After 45 days of incubation of Darien soil with 10 μg of labeled alachlor/mL, 65.0 and 40.6% of the ^{14}C was recovered in the autoclaved and unautoclaved samples, respectively. Of the ^{14}C recovered in the autoclaved sample, 36.2, 59.2, and 4.7% of the counts were found in the ethyl acetate, hexane–acetone, and aqueous fractions, respectively. In the sample that had not been autoclaved, 10.4, 14.5, and 75.1% of the radioactivity was in the ethyl acetate, hexane–acetone, and aqueous fractions, respectively. On the basis of thin-layer chromatography on silica gel sheets developed in toluene–methanol (95:5), only alachlor was present in the ethyl acetate and hexane–acetone fractions in both autoclaved and unautoclaved samples. Because of the increase in radioactivity in the aqueous fraction of the unautoclaved sample, this fraction was placed on a C_{18} column to separate alachlor from its metabolic products. All the ^{14}C present in the aqueous fraction eluted in the ether–methanol fractions (Figure 1B).

Enrichments mineralizing 1 or 100 μg of alachlor/mL could not be obtained from the six soils, whereas a mixed culture able to mineralize 100 μg of propachlor/mL was obtained. After 6 months of serial transfers to fresh media, the mixture contained a gram-positive, oxidase-negative coccus and a small gram-negative, oxidase-positive rod.

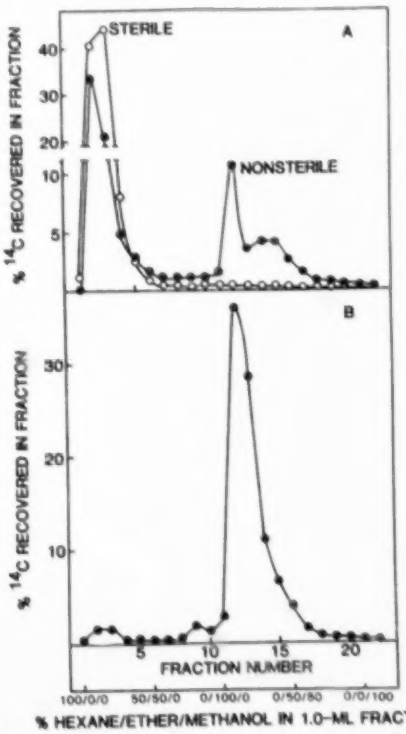

Figure 1. C_{18} elution profile of aqueous extracts of aquifer (A) and soil (B) samples.

The rod produced large yellow colonies on trypticase soy agar. When streaked on trypticase soy agar and reinoculated separately into the medium containing 100 μg of propachlor/mL and mineral salts, the separate isolates did not mineralize the ring carbons of propachlor. After inoculation of both isolates from trypticase soy agar into the propachlor–salts medium, mineralization of propachlor was evident but only about 50% of the time. However, if the enrichments were maintained in the propachlor–inorganic salts medium and transferred biweekly, high propachlor–mineralizing activity was sustained. Thus, when 25 mL

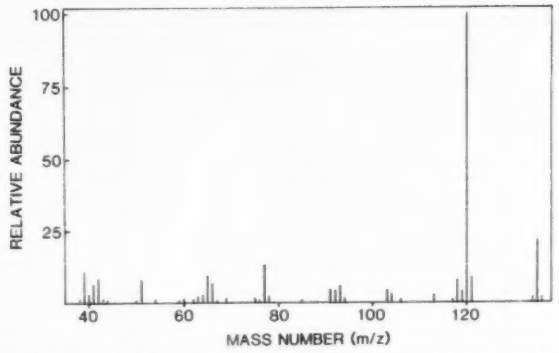

Figure 2. Mass spectrum of product identified as *N*-isopropylaniline.

of the inorganic salts solution containing 100 μg of propachlor/mL (0.25% of which was [^{14}C]propachlor) was inoculated with 0.2 mL of a 5-day-old mixed culture, 57.6% of the ring-labeled carbons of propachlor was converted to CO_2 in 52.5 h. Gas chromatographic analysis indicated that no propachlor remained in the medium after 52.5 h. No additional $^{14}CO_2$ was formed in the following 13 h.

To identify the products formed, triplicate cultures and uninoculated controls that had been incubated with 100 μg of propachlor/mL for 42 h, at which time 0–8% (depending on the experiment) of the propachlor had been converted to CO_2, were extracted with ethyl acetate and analyzed for products of metabolism by combined gas chromatography–mass spectrometry. A product was found that was identified as *N*-isopropylaniline (Figure 2). The values of m/z at 135, 120, 77, 51, and 39 are characteristic of $C_9H_{13}N$, $C_9H_{13}N - CH_3$, $C_6H_5^+$, $C_4H_3^+$, and $C_3H_3^+$, respectively.

DISCUSSION

Although the soils had received applications in the field of alachlor for one or two previous growing seasons or propachlor for up to three previous growing seasons, only a small percentage of the ring carbons of alachlor was converted to CO_2 under aerobic conditions during the 30-day period. Because the alachlor preparation was only 97% pure, much or, in some instances, all of the CO_2 evolved may be from a contaminant in the herbicide preparation. However, alachlor was converted to polar organic products in a soil that had received alachlor during two growing seasons. The conversion of alachlor to organic products with little or no CO_2 production from the ring carbons occurs in pure cultures of fungi (Chahal et al., 1976; Smith and Phillips, 1975; Tiedje and Hagedorn, 1975) and in a number of other environments not previously exposed to the herbicide (Novick and Alexander, 1985; Yu et al., 1975). Only about 40% of the [^{14}C]alachlor added to soil slurries could be recovered after a 45-day incubation. Chou (1977) reported that products of fungal metabolism of alachlor bind strongly to soil humic acids, and only part can be recovered by extraction. The poor recovery of ^{14}C in autoclaved samples (60%) may be a result of the strong binding of the parent compound to soil organic matter (Beestman and Deming, 1974). Products were not formed in autoclaved samples, however.

Although alachlor does not move readily through the soil, low levels have been detected in runoff water (Baker and Laflen, 1979; Wu, 1980; Wu et al., 1983), and alachlor and demethoxymethylalachlor have been found in ground water samples (Peterson, 1984). The results of the present study indicate that trace levels of alachlor may be converted to polar products in ground water aquifers but mineralization may not occur.

Propachlor has been reported to be slowly or not mineralized in soils (Kaufman et al., 1971; Lee et al., 1982), sewage, and lake water samples (Novick and Alexander, 1985). In all cases, organic products of propachlor metabolism were found. In the present study, however, microorganisms in suspensions of the four mineral soils and in the two samples of organic soil extensively mineralized propachlor. About 60% of ring-labeled propachlor was mineralized in 30 days in suspensions of muck soils treated in the field with this pesticide in the three previous growing seasons. It is not known whether the high mineralization rates in the muck soils are a result of the pretreatment, the high organic matter content or active microfloras of these soils, or some other factor. The data show that two bacteria together are able to mineralize the ring carbons of propachlor. It is not clear how these two isolates interact in the process, but it is possible that *N*-isopropylaniline is an intermediate in this interaction. Lee et al. (1982) had earlier found that *N*-isopropylaniline, *N*-isopropylacetanilide, *N*-(1-hydroxyisopropyl)acetanilide, and *N*-isopropyl-2-acetoxyacetanilide were generated from propachlor in soil suspensions; only *N*-isopropylaniline was found as a product of the mixed culture in the present investigation.

The results of this study suggest that alachlor and its metabolites may not be rapidly mineralized in alachlor-pretreated soils but may persist for long periods. Because of this persistence, they may be transported to other sites, such as by eroding soil particles. Should they be leached into ground water aquifers, these compounds may be further metabolized to organic products, albeit very slowly. In contrast, the data show appreciable mineralization of propachlor in soil suspensions, although differences exist among soils. Little or no mineralization of propachlor and alachlor was evident in the aquifer material tested. Further study is needed to determine whether the major differences in the susceptibility of these acetanilide herbicides to mineralization are reflected in product accumulation in the field.

Registry No. Alachlor, 15972-60-8; propachlor, 1918-16-7; PhUHPr-*i*, 768-52-5.

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